Europäisches Patentamt
European Patent Office
Office européen des brevets

Bescheinigung Certificate Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03023539.4

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 03023539.4
Demande no:

Anmeldetag:
Date of filing: 15.10.03
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Munich Biotech AG
Forstenriederstrasse 10
82061 Neuried
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Method of administering cationic liposomes comprising an active drug

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

EP/30.05.03/EP 03012449

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K9/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

WEICKMANN & WEICKMANN

Patentanwälte
European Patent Attorneys - European Trademark Attorneys

DIPL.-ING. H. WEICKMANN (bis
DIPL.-ING. F. A. WEICKMANN
DIPL.-CHEM. B. HUBER
DR.-ING. H. LISKA
DIPL.-PHYS. DR. J. PRECHTEL
DIPL.-CHEM. DR. B. BÖHM
DIPL.-CHEM. DR. W. WEISS
DIPL.-PHYS. DR. J. TIESMEYER
DIPL.-PHYS. DR. M. HERZOG
DIPL.-PHYS. B. RUTTENSPERGER
DIPL.-PHYS. DR.-ING. V. JORDAN
DIPL.-CHEM. DR. M. DEY
DIPL.-FORSTW. DR. J. LACHNIT

Our Reference:

30852P EP-1/WWkt

Applicant:

Munich Biotech AG
Forstenrieder Str. 10

82061 Neuried

Method of Administering Cationic Liposomes Comprising an Active Drug

---

Postfach 860 820, 81635 München, Deutschland, Tel. (089) 45563 0, Fax (089) 45563 999, email@weickmann.de

Method of Administering Cationic Liposomes Comprising an Active Drug

Description

The present invention relates to the use of pharmaceutical preparations comprising paclitaxel for administration to a human patient in need thereof.

The use of antimitotic drugs, such as taxanes, as therapeutic agents for human patients suffering from diseases which are connected with enhanced mitosis are well known in the art.

Paclitaxel has a unique mechanism of action and a broad spectrum of antiproliferative activity because paclitaxel binds to microtubules and promotes tubulin polymerisation and stabilizes the assembled microtubules. As a result, paclitaxel blocks the cell cycle at prophase resulting in an accumulation of cells in the G2/M phase.

Unfortunately, paclitaxel has extreme low solubility in water, which makes it difficult to provide a suitable dosage form. Currently, paclitaxel is formulated and administered in a vehicle containing Cremophor EL (a polyethoxylated castor oil) and ethanol in a 50:50 (vol/vol) ratio. This solution is diluted 1:10 in saline before being administered to humans. However, various severe side reactions, such as hypersensitivity and hypertensive reactions, nephrotoxicity and neurotoxicity, for example, have been reported in patients due to Cremophor EL formulation.

Further even much cardiac [illegible] other antitumor drugs) is a potent

dividing tumor cells gain the capacity to overcome the growth inhibitory effect of a selected anti-cancer drug ({Vogelstein, 1988 #8}, {Kerbel, 1991 #9}). This capacity is usually not limited to a single drug (first line) but extends to other drugs which are used after development of the first resistance. Hence, this phenomenon is called multi drug resistance (MDR). As the number of available and approved anti-neoplastic drugs is very limited for many cancer types, many patients succumb since their cancer tissues express MDR. The obvious problem, therefore, is to find methods and means to kill drug-resistant tumors, especially drug resistant cells, which are already resistant against the respective drug.

A number of approaches were taken to deal with the above mentioned problems. The conventional strategy is to increase doses up to the maximal tolerated dose (MTD) and attempt to eradicate all tumor cells as quickly and completely as possible ({Schünemann, 1999 #10}, {Heidemann, 1997 #11}). It is obvious that this strategy causes severe side effects and can not be extended to longer periods. Therefore, this treatment schedule consists of cycles of one short treatment period (usually 1 day – 1 week) at MTD and a treatment-free interval of several weeks (usually 3-4 weeks), to allow the patient to recover from the obligatory side effects ({Schünemann, 1999 #10}, {Heidemann, 1997 #11}, {Romanini, 2003 #4}). In many instances, tumor growth can also restart during these drug-free periods. Most importantly, this approach fails in many patients where tumor cells develop a high level of resistance which enables them to accommodate with drug concentrations at the MTD. The patients become therapy refractory.

The most common solution is to start treatment with a second drug ({Blom, 1996 #5}, {Awada, 2002 #2}, {Seidman, 2003 #3}, {Heinemann, 2003 #12}, {Thigpen, 2003 #13}). In the best case, the second line treatment is successful and the patient is cured. A common experience however is that tumors only respond for a certain time leading to a temporary regression of the tumor. After that, tumors become also resistant to the second drug.

Continuing with this strategy leads to development of multi drug resistant tumors which are finally refractory to all available anti-cancer drugs ({Blom, 1996 #5}, {Seidman, 2003 #3}, {Thigpen, 2003 #13}).

Another possibility is to treat patients immediately with a combination of 2 or more drugs ({Heinemann, 2003 #12}, {Kuenen, 2002 #14}, {Sledge, 2003 #15}, {Ozols, 2003 #7}, {Reck, 2003 #17}, {Romanini, 2003 #4}). This strategy can be more successful as it decreases the likelihood for development of a double drug resistance. However, this strategy needs to explore time and cost intensively suitable drug combinations. A second disadvantage is that the side effects may also increase ({Kuenen, 2002 #14}, {Ozols, 2003 #7}). The therapeutic window concomitantly becomes small and the toxic effects may overlay the envisioned therapeutic benefit. Also in this case, multi drug resistance may develop and the therapy becomes ineffective ({Zimpfer-Rechner, 2003 #18}, {Sledge, 2003 #15}, {Sledge, 2003 #16}, {Ozols, 2003 #7}).

The consequence of the negative experiences with such traditional treatment strategies is to develop more and more new drugs to extend the above described treatment options. Obviously, it is a very time and cost intensive race for more potent drugs which will eventually lead in many cases to therapy refractory tumors. In recent years, this recognition has led to a new approach to circumvent tumor resistance. It is based on the assumption that the MDR is caused by overexpression of enzymes which enable cells to expel chemotherapeutic drugs. The most famous member of this category of enzymes is called p-glycoprotein (p-gp). It is located in the cytoplasmic membrane and exports in an ATP-driven way ({Nobmann, 2001 #19}, [illegible] compounds like vechicine or doxorubicin ({Hamer,

studies, however, revealed unsatisfactory results, possibly due to to low specific activity ({Thomas, 2003 #20}, {Kohler, 2003 #24}). The further research led to a second generation of compounds which again were found not to be clinically applicable ({Leonard, 2002 #25}, {Thomas, 2003 #20}). Today a few substances of the third generation, one known as tariquidar, are in clinical trials ({Agrawal, 2003 #26}, {Callies, 2003 #27}). The usefulness and broad applicability of these compounds is, however, still unclear ({Leonard, 2002 #25}, {Thomas, 2003 #20}). Even though much improved in comparison to first generation chemosensitizers, third generation compounds also cause side effects and may have unforeseen consequences for the whole body. Extensive clinical testing is needed and it is so far uncertain if such approaches can become general practice in the future ({Leonard, 2002 #25}, {Thomas, 2003 #20}).

Different delivery systems have been used to enhance the effect of paclitaxel and/or reduce toxicity. Liposomes are one of many carriers that have been developed to enhance aqueous solubility and thus efficiency, combined with less toxicity.

U.S. Pat. No. 5,648,090, U.S. Pat. No. 5,424,073 and U.S. Pat. No. 6,146,659 (Rahman et al.) provide a liposomal encapsulated paclitaxel for a method for treating cancer in mammals. These patents disclose a method of administering to the host a pharmaceutical composition of a therapeutically effective amount of liposomes which include a liposome forming material, cardiolipin, and an agent such as paclitaxel, or an antineoplastic derivative of paclitaxel, or a mixture thereof, with a pharmaceutically acceptable excipient. In U.S. Pat. No. 6,146,659, a method of administering a taxane to a patient is provided by administering taxane over a period of less than an hour in an amount from about 75 to 300 mg/m^2, wherein the taxane is liposomally encapsulated. The liposomes disclosed therein are negatively charged.

Since the disclosure of McDonald et al., U.S. Pat. No. 5,837,283, it is known that positively charged liposomes specifically target angiogenic endothelial cells. Clinical data on human patients are not presented.

Thus, the problem underlying the present invention was to provide a method of administering paclitaxel to a subject in need thereof in a therapeutically effective amount without severe side effects.

Surprisingly, it was found that administration of a cationic liposomal preparation having a high content of paclitaxel as an active ingredient selectively affects angiogenic endothelial cells in a human patient in need thereof. Thus, the invention comprises administering to said patient a cationic liposomal preparation comprising from about 30 mole% to about 98 mole% cationic lipid, paclitaxel in an amount of at least about 2 mole% and neutral lipids from about 0 mole % to about 70 mole% at a monthly dose of about 0.25 mg up to about 60 mg of paclitaxel / kg body weight of said patient.

A further aspect of the invention comprises administering to a human patient in need thereof a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% at a monthly dose of about 0.25 mg up to about 60 mg of paclitaxel / kg body weight of said patient.

The above formulations are particularly suitable for the prevention and/or treatment of multi drug resistant tumors and/or tumor metastases, optionally in combination with other treatment protocols.

- improved efficacy
- avoiding multi drug resistance (different target)
- affecting drug resistance by killing resistant cells directly
- affecting metastasis
- lower side effects compared to traditional chemotherapy or with neutral or anionic liposomes
- reduction of disease related pain
- improvement of quality of life
- stabilization of body weight during treatment
- synergistic effects with traditional therapy regimes

The present pharmaceutical composition can be administered at a monthly dose of about 0.25 mg up to about 60 mg of liposomal paclitaxel / kg body weight (bw) of a patient, preferably of about 0.5 mg up to about 30 mg of liposomal paclitaxel / kg bw and more preferably of about 1.0 mg up to about 15 mg of liposomal paclitaxel / kg bw. On an average, a human patient has about 70 kg body weight and is about 172 cm tall.

The dose scheme can range from a plurality of times daily to a plurality of times during a month period, each of said times being separated by an interval of between one day and 3 weeks. The total treatment period is preferably at least one month.

The present pharmaceutical composition can be administered at a single unit dose scheme of about 0.01 to 10 mg liposomal paclitaxel per kg body weight. In a preferred embodiment of the present invention about 0.05 to about 5 mg liposomal paclitaxel per kg of body weight is administered at a single unit dose. Preferably, 0.1 to 2.5 mg liposomal paclitaxel per kg of body weight per single unit dose is administered.

The suitable dose of liposomal paclitaxel for application to a human patient is in an amount of about 0.01 to 2.5, preferably 0.02 to 1.0, and more preferably

0.05 to 0.5 mg / kg bw at least once a day, e.g. twice, three times or more each day; about 0.01 to 5.0, preferably 0.02 to 2.5 and more preferably 0.05 to 1.0 mg / kg bw every other day; about 0.01 to 10, preferably 0.02 to 5.0 and more preferably 0.05 to 2.5 mg / kg bw once a week.

The monthly dose is preferably administered in a plurality of single dose units. During the treatment interval the dose units and the dose intervals may remain constant. On the other hand, the dose units may be increased during the treatment interval, e.g. beginning with a starting dose and escalating in one or several steps to a consolidation dose, which may be 3 or 4 or more times higher than the starting dose. Additionally or alternatively, the treatment interval between single doses may be altered, e.g. decreased or increased during the treatment period.

In an even further aspect, the cationic liposomal preparation of the present invention comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, preferably to about 98 mole% cationic lipid, paclitaxel in an amount of at least about 0.1 mole%, preferably of at least about 2 mole%; and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryotherapy.

In a preferred embodiment, the liposomal preparation comprises paclitaxel in an amount of about 0.1 mole%, particularly of about 2 mole%, to about 8 mole%, preferably in an amount of about 0.5 mole%, particularly of about 2 mole%, more preferably in an amount of about 1 mole% to

The liposomal preparation of the present invention comprises cationic lipids in an amount of about 30 mole% to about 99.9 mole%, particularly to about 70 mole%, preferably from about 40 mole% to about 60 mole% and most preferably from about 45 mole%, to about 55 mole% and are characterized by having a positive zeta potential in about 0.05 M KCl solution at about pH 7.5 at room temperature.

The preferred cationic lipids of the liposomal preparation are N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium salts, e.g. the methylsulfate (DOTAP). Other useful lipids for the present invention may include: DDAB, dimethyldioctadecyl ammonium bromide; 1,2-diacyloxy-3-trimethylammonium propanes, (including but not limited to: dioleoyl, dimyristoyl, dilauroyl, dipalmitoyl and distearoyl; also two different acyl chain can be linked to the glycerol backbone); N-[1-(2,3-dioloyloxy)propyl]-N,N-dimethyl amine (DODAP); 1,2-diacyloxy-3-dimethylammonium propanes, (including but not limited to: dioleoyl, dimyristoyl, dilauroyl, dipalmitoyl and distearoyl; also two different acyl chain can be linked to the glycerol backbone); N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA); 1,2-dialkyloxy-3-dimethylammonium propanes, (including but not limited to: dioleyl, dimyristyl, dilauryl, dipalmityl and distearyl; also two different alkyl chain can be linked to the glycerol backbone); dioctadecylamidoglycylspermine (DOGS); 3β-[N-(N',N'-dimethylaminoethane) carbamoyl]cholesterol (DC-Chol); 2,3-dioleoyloxy-N-(2-(sperminecarboxamido)-ethyl)-N,N-dimethyl-1-propanaminium trifluoro-acetate (DOSPA); β-alanyl cholesterol; cetyl trimethyl ammonium bromide (CTAB); diC14-amidine; N-*tert*-butyl-N'-tetradecyl-3-tetradecylaminopropionamidine; 14Dea2; N-(alpha-trimethylammonioacetyl) didodecyl-D-glutamate chloride (TMAG); O,O'-ditetradecanoyl-N-(trimethylammonioacetyl)diethanolamine chloride; 1,3-dioleoyloxy-2-(6-carboxy-spermyl)-propylamide (DOSPER); N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxylethyl)-2,3-dioleoyloxy-1,4-butanediammonium iodide; 1-[2-(acyloxy) ethyl]2-alkyl(alkenyl)-3-(2-hydroxyethyl)-imidazolinium chloride derivatives as

described by Solodin et al. (1995) Biochem. 43:13537-13544, such as 1-[2-(9 (Z)-octadecenoyloxy)ethyl]-2-(8(Z)-heptadecenyl-3-(2-hydroxyethyl) imidazolinium chloride (DOTIM), 1-[2-(hexadecanoyloxy)ethyl]-2-pentadecyl-3-(2-hydroxyethyl)imidazolinium chloride (DPTIM), 2,3-dialkyloxypropyl quaternary ammonium compound derivatives, containing a hydroxyalkyl moiety on the quaternary amine, as described e.g. by Felgner et al. [Felgner et al. *J. Biol. Chem.* 1994, 269, 2550-2561] such as: 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), 1,2-dioleyloxypropyl-3-dimetyl-hydroxypropyl ammonium bromide (DORIE-HP), 1,2-dioleyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-Hpe), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxylethyl ammonium bromide (DMRIE), 1,2-dipalmityloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE), 1,2-disteryloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE); cationic esters of acyl carnitines as reported by Santaniello et al. [US5498633]; cationic triesters of phospahtidylcholine, i.e. 1,2-diacyl-sn-glycerol-3-ethylphosphocholines, where the hydrocarbon chains can be saturated or unsaturated and branched or non-branched with a chain length from C_{12} to C_{24}, the two acyl chains being not necessarily identical.

In a preferred embodiment, the liposomal preparation optionally comprises at least one neutral lipid. Neutral lipids are lipids which have a neutral net charge. These can be selected from sterols or lipids such as cholesterol, phospholipids, lysolipids, lysophospholipids, sphingolipids or pegylated lipids with a neutral net charge. Useful neutral lipids thereby include: phosphatidylserine, phosphatidylglycerol, phosphatidylinositol (not limited to [illegible] containing a terminal acid group for

also have two different fatty acids. Preferably the further lipids are in the liquid crystalline state at room temperature and they are miscible (i.e. a uniform phase can be formed and no phase separation or domain formation occurs) with the used cationic lipid, in the ratio as they are applied. In a preferred embodiment the neutral lipid is DOPC.

In a further preferred embodiment, the liposomal preparation comprises optionally neutral lipids, preferably DOPC in an amount of about 30 mole% to about 70 mole%, preferably from about 40 mole% to about 60 mole% and more preferably from about 45 mole% to about 55 mole%,

It is a further object of the present invention that the cationic liposome preparation which is used therein can be dehydrated, stored for extended periods of time while dehydrated, and then rehydrated when and where it is to be used, without losing a substantial portion of its contents during the dehydration, storage and rehydration processes. To achieve the latter, one or more protective agents, such as cryoprotectants, may be present. Thus, the inventive cationic liposome preparation preferably comprises a cryoprotectant, wherein the cryoprotectant is selected from a sugar or an alcohol or a combination thereof. Preferably, the cryoprotectant is selected from trehalose, maltose, sucrose, glucose, lactose, dextran, mannitol or sorbitol.

In a further preferred embodiment, the liposomal preparation comprises trehalose in the range of about 5 % (m/v) to about 15 % (m/v) with respect to the total volume of the preparation.

The formulation of the cationic liposomes of the present invention may vary. In a preferred embodiment the molar ratio is 50:47:3 mole% of DOTAP, DOPC and paclitaxel.

Liposomes of various sizes are useful in the present invention. In a preferred embodiment of the present invention cationic liposomes have an average particle diameter from about 50 to about 500 nm, preferably from about 100 nm to about 300 nm.

The present liposome compositions can be administered systemically, preferably intravenously.

The cationic liposomes of the present invention may be used to treat any form of a condition associated with increased angiogenesis, such as cancer. The pharmaceutical composition of the present invention is particularly advantageous in treating tumors in human patients such as bladder cancer, breast cancer, colorectal cancer, endometrial cancer, leukaemia, lung cancer, lymphoma, melanoma, non-small-cell lung cancer, ovarian cancer, prostate cancer and to childhood cancers such as brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma, ependymoma, Ewing's sarcoma/family of tumors, germ cell tumor, extracranial, Hodgkin's disease, leukaemia, acute lymphoblastic, leukaemia, acute myeloid, liver cancer, medulloblastoma, neuroblastoma, non-Hodgkin's lymphoma, osteosarcoma/malignant fibrous histiocytoma of bone, retinoblastoma, rhabdomyosarcoma, soft tissue sarcoma, supratentorial primitive neuroectodermal and pineal tumors, unusual childhood cancers, visual pathway and hypothalamic glioma, Wilms' Tumor and other childhood kidney tumors and to less common cancers including acute lymphocytic leukaemia, adult acute myeloid leukaemia, adult non-Hodgkin's lymphoma, brain tumor, cervical cancer, childhood cancers, childhood sarcoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, esophageal cancer, hairy cell [illegible] multiple myeloma, neuroblastoma,

progression, or may lead to a partial or complete remission. Further conditions may be wound healing or an inflammatory disease or a chronic inflammatory disease such as rheumatoid arthritis, dermatitis, endometriosis or psoriasis.

Surprisingly, it was found that active agents loaded into cationic liposomes act directly against endothelial or non-endothelial drug resistant cells, particularly drug resistant endothelial or non-endothelial tumor cells. Thus, the recent findings define non-endothelial drug resistant cells as a further target and thus enhance the anti-tumor applications of cationic liposomes. This is an additional aspect of the present invention.

Thus it is a further object of the present invention to use a cationic liposomal preparation comprising an active agent for the manufacture of a medicament against endothelial or non-endothelial drug resistant cells. The present invention also provides a method of administering a cationic liposomal preparation comprising an active agent to drug resistant cells of a subject in need thereof in a therapeutically effective amount to affect a disease such as cancer.

The cationic liposomal preparation of this aspect of the present invention comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, particularly to about 98 mole%, an active agent in an amount of at least about 0.1 mole%, particularly of at least about 2 mole%, and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for affecting drug resistant cells such that a disease associated with or accompanied by the occurrence of drug resistant cells is relieved (causing regression) or eventually cured.

It is a further surprising finding within the present invention that cationic liposomes comprising an active agent act alone or in combination with at least one other treatment therapy against metastasis formation.

Thus, it is a further object of the present invention to use a cationic liposomal preparation comprising an active agent for preparing a medicament against metastasis. The present invention also provides a method of administering a cationic liposomal preparation comprising an active agent to a subject in need thereof in a therapeutically effective amount to affect onset and/or progression of metastasis formation such as delaying and/or avoiding a metastatic disease. The term "affecting" as used herein generally means that a desired pharmacologic and/or physiologic effect is obtained, such as delaying and/or avoiding the onset and/or progression of a disease. In a preferred embodiment, the present invention is used for delaying and/or avoiding liver metastasis formation.

In an even further aspect, the cationic liposomal preparation of the present invention comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, particularly to about 98 mole%, a first active agent, e.g. paclitaxel in an amount of at least about 0.1 mole%, particularly of at least about 2 mole%, and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one second active agent, e.g. a second non-liposomal active agent and/or heat and/or radiation and/or cryotherapy for delaying and/or avoiding metastasis formation.

The active agent loaded into the cationic liposomal preparation, e.g. an active agent for a monotherapy or the first active agent for a combination therapy, can be selected from a cytotoxic or cytostatic substance such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent or immunologically active substance. In a more preferred embodiment, the

docetaxel, camptothecin or any derivative thereof.

Thus, in a preferred embodiment of the present invention said liposomal preparation comprises a taxane, preferably paclitaxel or docetaxel or a derivative thereof in an amount of about 0.1 to about 20 mol%, preferably in an amount of about 0.5 mole% to about 10 mole%, more preferably in an amount of about 1 mole% to about 5 mole% and most preferably in an amount of about 2 mole% to about 4 mole%.

The at least one second active agent may be a cytotoxic or cytostatic substance as described above, such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent, an immunological active substance, a compound that reduces or eliminates hypersensitivity reactions or a chemosensitizer. Preferably, the at least one second active agent is present in a non-liposomal formulation. Further, it is preferred that the first and the second active agents are different.

In a preferred embodiment, the active agents are selected from antineoplastic agents such as: antimitotic agents like paclitaxel (Taxol), alkylating agents such as platinum containing compounds like cisplatin, carboplatin, DNA topoisomerase inhibiting agents like camptothecin or doxorubicin, RNA/DNA antimetabolites such as 5-fluorouracil or gemcitabine and other compounds having antitumor activity. Especially preferred are combination therapies with cisplatin or carboplatin or with 5-fluorouracil or with gemcitabine.

In a further preferred embodiment, the compound that reduces or eliminates hypersensitivity reactions is selected from the group comprising (but not limited to) steroids, antihistamines, H2 receptor antagonists, and combinations thereof in a sufficient amount to prevent fatal anaphylactic reactions. In an even more preferred embodiment the compound is selected from the group comprising Ranitidine, Dexamethasone, Diphenhydramine, Famotidine, Hydrocortisone, Clemastine, Cimetidine, Prednisolone, Prednison, Chlorpheniramine, Chlorphenamine, Dimethindene maleate,

Indomethazine and Promethazine or any derivative thereof.

In a preferred embodiment, the chemosensitizer is selected from the group comprising (but not limited to) cell cycle modulators, substances that revert a drug resistance like verapamil, vasoactive substances like anti-hypertensive drugs, substances that modify the charge-related interaction of cationic liposomes with blood components like protamine.

Figure Legends

Figure 1: Tumor size of L3.6pl pancreatic tumors 19 days after start of treatment. Treatment with 10% trehalose, Taxol, DOTAP/DOPC/paclitaxel 50:47:3 mole % (MBT-0206), Gemzar (gemcitabine) and the combination of both MBT-0206 and Gemzar started 8 days after tumor cell inoculation. Gemzar was applied i.p. at a dose of 100 mg/kg bw twice a week (Mon, Thu). Taxol and MBT-0206 were applied i.v. on a Mon, Wed, Fri schedule at a paclitaxel dose of 5 mg/kg bw. The combination group received both MBT-0206 and Gemzar with the respective schedule. Tumors were measured by palpation with a calliper on day 23 and 27. Mean ± SEM; n = 9 per group.

Figure 2: Metastases at day 19 after start of treatment. Treatment with 10% trehalose, Taxol, MBT-0206, Gemzar (gemcitabine) and the combination of both MBT-0206 and Gemzar started at day 8 after tumor cell inoculation. Gemzar was applied i.p. at a dose of 100 mg/kg bw twice a week (Mon, Thu). Taxol and MBT-0206 were applied i.v. on a Mon, Wed, Fri schedule at a paclitaxel dose of 5 mg/kg bw. The combination group received both MBT-0206 and Gemzar with the respective schedule, n = 9 per group.

Figure 3 - 5: The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4 x 10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 10-11 concentrations of the respective drug formulation were added for 72 h to cover the range depicted in the respective graphs. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of mitochondrial dehydrogenases.

Figure 6 - 7: The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4 x 10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 10-11 concentrations of the respective drug formulation were added for

72 h to cover the range depicted in the respective graphs. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of mitochondrial dehydrogenases.

Figure 8: The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4 x 10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 11 concentrations of the respective drug formulation were added for 72 h to cover the range depicted in the respective graph. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of mitochondrial dehydrogenases.

The following examples should be illustrative only but are not meant to be limiting to the scope of the invention. Other generic and specific configurations will be apparent to those skilled in the art.

Examples

1. Human Therapy Treatment Protocol

This example is concerned with human treatment protocols using the formulations disclosed. Treatment will be of use preventing and/or treating various human diseases and disorders associated with enhanced angiogenic activity. It is considered to be particularly useful in anti-tumor therapy, for example, in treating patients with solid tumors and hematological malignancies or in therapy against a variety of chronic inflammatory diseases such as rheumatoid arthritis or psoriasis.

is killed without directly targeting the tumor cells in any manner. Other classes of diseases and/or abnormalities may be treated by directly targeting angiogenic endothelial cells and by directly targeting the tissue or cells involved in the abnormality.

In an other application, drug resistant cells such as drug resistant cancer cells or highly proliferative synoviocytes in rheumatoid arthritis can be affected directly.

The various elements of conducting a clinical trial, including patient treatment and monitoring, will be known to those skilled in the art in light of the present disclosure.

For regulatory approval purposes, it is contemplated that patients chosen for a study would have failed to respond to at least one course of conventional therapy and would have objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Such patients would also have no history of cardiac or renal disease and any chemotherapy should be stopped at least 2 weeks before entry into the study.

Prior to application, the formulation can be reconstituted in an aqueous solution in the event that the formulation was freeze dried. As outlined above, the required application volume is calculated from the patient's body weight and the dose schedule.

The disclosed formulations may be administered over a short infusion time. The infusion given at any dose level should be dependent upon the toxicity achieved after each. Thus, if Grade II toxicity was reached after any single infusion, or at a particular period of time for a steady rate infusion, further doses should be withheld or the steady rate infusion stopped unless toxicity improved. Increasing doses should be administered to groups of patients

until approximately 60% of patients showed unacceptable Grade III or IV toxicity in any category. Doses that are 2/3 of this value would be defined as the safe dose.

Physical examination, tumor measurements and laboratory tests should, of course, be performed before treatment and at intervals of about 3-4 weeks later. Laboratory tests should include complete blood cell counts, serum creatinine, creatine kinase, electrolytes, urea, nitrogen, SGOT, bilirubin, albumin and total serum protein.

Clinical responses may be defined by acceptable measure or changes in laboratory values e.g. tumor markers. For example, a complete response may be defined by the disappearance of all measurable disease for at least a month, whereas a partial response may be defined by a 50% or greater reduction.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those skilled in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed [illegible] spirit, scope and concept of the invention as defined by the [illegible]

any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the FDA Office of Biologics standards.

The present invention includes a method of delivery of a pharmaceutically effective amount of the inventive formulation of an active agent to a target site such as an angiogenic vascular target site of a subject in need thereof. A "subject in need thereof" refers to a mammal, e. g. a human.

The route of administration preferably comprises peritoneal or parenteral administration.

For use with the present invention the "pharmacologically effective amount" of a compound administered to a subject in need thereof will vary depending on a wide range of factors. The amount of the compound will depend upon the size, age, sex, weight, and condition of the patient, as well as the potency of the substance being administered. Having indicated that there is considerable variability in terms of dosing, it is believed that those skilled in the art can, using the present disclosure, readily determine appropriate dosing by first administering extremely small amounts and incrementally increasing the dose until the desired results are obtained. Although the amount of the dose will vary greatly based on factors as described above, in general, the present invention makes it possible to administer substantially smaller amounts of any substance as compared with delivery systems which only target the pathologic tissue e. g., target the tumor cells themselves.

2. Mono-therapy protocols

Study No.	Indication
CTLP01	Prostate Cancer
CTLP05	Gastro-Intestinal Cancer

Dosing

Study No.	Dosages
CTLP01	0.06 mg/kg, 0.25 mg/kg, 1.0 mg/kg or 1.5 mg/kg (mg liposomal paclitaxel / kg)
CTLP05	0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg or 1.5 mg/kg (mg liposomal paclitaxel / kg)

Standard formulation liposomal paclitaxel

50 mol% DOTAP : 47 mol% DOPC : 3 mol% Paclitaxel

Treatment schedule for liposomal paclitaxel in ongoing studies

Study No.	Schedule	No. of applications
CTLP01	3 times a week	N=3
CTLP05	3 times a week with 3 weeks interval each	N=6

Efficacy

Response will be evaluated according to the WHO or RECIST criteria.

3. Combination therapy protocols

Dosing

Study No.	Dosages
CTLP04	liposomal paclitaxel, 1.5 mg/kg and Carboplatin (2mg/ml/min i.v. For 15 min once weekly)
CTLP06	liposomal paclitaxel, 0.5 or 1.0 mg/kg alone, 0.5 or 1.0 mg/kg liposomal paclitaxel and 5-fluorouracil (2000 mg/m²).

Standard formulation liposomal paclitaxel

50 mol% DOTAP : 47 mol% DOPC : 3 mol% Paclitaxel

Treatment schedule for liposomal paclitaxel in ongoing studies

Study No.	Schedule	No. of applications
CTLP04	once weekly	N=14
CTLP06	daily with one week terval each	N=14

A further planned study comprises administration of liposomal paclitaxel, e.g. 0.5 or 1.0 or 1.5 mg/kg and gemcitabine, e.g. 1000 mg/m² once weekly for three weeks followed by one week without treatment, preferably for an interval of at least one year.

The treatment schedule for liposomal paclitaxel will be as descibed above for ongoing studies.

Efficacy

Response will be evaluated according to the WHO or RECIST criteria.

4. Case report #1

Patient:

- 49 years old patient with large therapy resistant recidivism of a mucoepidermoidal carcinoma of the larynx
- metastases cervical, supraclavicular, axillar, mediastinal and pulmonal
- 5 years after first tumor resection, neck tumor dissection and adjuvant radiotherapy
- after repeated therapy of recidivism with multiple resections, plastic surgery, radiotherapy and chemotherapy
-

Dosing schedule:

- MBT-0206: 50/47/3 (DOTAP/DOPC/paclitaxel)
- Application of 0.06, 0.25, 0.5 and 1.0 mg liposomal paclitaxel / kg bw, i.v.
- One cycle of 3 times a week (on day 1, 3 and 5)

Results:

- Good tolerance while monitoring cardiovascular, pulmonary and serological parameter during and after infusion
- No signs of acute of chronic toxicity
- Reduction of tumor blood circulation
- Strongly reduced progression of tumor growth during 3 months

5. Case report #2

One patient with liver cell carcinoma, who had disease progression after

or peripheral intravenous infusion over a period of 2-4h. The infusion rate has been increased slowly up to a maximum speed of 2,5 ml/min. Premedication depended on the patient's sex, age, condition. In the specific case of the above mentioned patient it has been given dexamethasone and an antihistamine.

MBT-0206 has been administered once weekly with a dose escalation schedule, beginning with 2 times 0.25 mg liposomal paclitaxel / kg bw, 1 times 0.5 mg liposomal paclitaxel / kg bw) and than a consolidation dose of 19 times 1.0 mg liposomal paclitaxel / kg bw. This treatment is after 22 weekly administrations still ongoing and up to now no adverse drug reactions have been reported. Besides the favourable safety profile the last evaluation of tumor size, which has been performed by CT-Scan of the liver, showed stable disease.

6. Case report #3

In another case a prostate cancer patient who became refractory to hormone therapy, has been treated with 1.0 mg liposomal paclitaxel / kg bw, 3 times weekly every third day under the same conditions of preparation and administration as described above. The premedication contained dexamethasone and antihistamines. The accumulated dose of liposomal paclitaxel for this patient in 7 days was 3.0 mg liposomal paclitaxel / kg bw.

7. Low dosing schedule with liposomal paclitaxel

Immortalised endothelial cells (EA.hy926) are seeded into 24-well plates (4 x 10^4 cells per well) and grown over night. The following day, 9 wells are treated for 1 h with the low dose of 51.2 ng/ml liposomal paclitaxel (60 nM) formulated as MBT-0206. In addition, 3 wells per formulation are treated with the high dose of 153.7 ng/ml (180 nM) liposomal paclitaxel formulated as MBT-0206 for 1 h and 3 wells remain untreated. Approximately 24 h later, 6

of the 9 low dose-treated wells are again treated with the same low doses of MBT-0206 for 1 h (i.e. 2x treatment groups). Again 24 h later, 3 of these 6 two times -treated wells are treated for the third time with 51.2 ng/ml paclitaxel formulated as MBT-0206 for 1h (3 x treatment groups). Approximately 96 h after this third treatment, the cell viability of all wells is quantitated. For this purpose, an assay which measures the activity of mitochondrial dehydrogenases using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is applied according to standard protocols (e.g. {Lindl, 1994 #28} with slight modifications).

The results demonstrate that the viability of cells treated for 3 times with a low dose of MBT-0206 is at least as strongly reduced as the viability of cells treated only one time with a high dose. Cells treated one time or two times with the low dose of MBT-0206 exhibit a somewhat increased viability which is, however, reduced in comparison to untreated cells.

Conclusion

Treatments with high doses of MBT-0206 can be replaced by using low doses at a higher frequency. There is a correlation between treatment density (no. of treatments per week) and treatment efficacy. Three weekly treatments with low doses were superior to 1 or 2 weekly treatments. This optimised dosing regimen potentially reduces toxic side effects caused by high dose treatments.

8. Anti-tumor efficacy of MBT-0206 in combination with gemcitabine (50 mg/kg) in L3.6pl pancreatic tumors

characterization of metastatic variants from human pancreatic adenocarcinoma by using orthotopic implantation in nude mice. Neoplasia 1999; 1(1):50-62.)

Supplier: Charles River France

Treatment

Treatment start: Day 7 after tumor inoculation

Last treatment: Day 26 after tumor inoculation

Dose: MBT-0206 with 5 mg paclitaxel / kg bw per application
Taxol at 5 mg paclitaxel / kg bw per application
Gemcitabine (Gemzar) at 50 mg / kg per application

Schedule: MBT-0206, Taxol, trehalose: d 7, 10, 12, 14, 17, 19, 21, 24, 26
Gemcitabine: d 7, 11, 14, 18, 21, 25
Combination: combined mono treatments

Application route: MBT-0206, Taxol, trehalose: i. v. bolus into tail vein
Gemcitabine: i. p. bolus

Groups (n = 9; n = 2-5 at day 26, 28)	Treatment
Trehalose, Taxol, MBT-0206	d 7, 10, 12, 14, 17, 19, 21, 24, 26
Gemcitabine [50 mg/kg]	d 7, 11, 14, 18, 21, 25
MBT-0206 [5 mg /kg bw] +	d 7, 10, 12, 14, 17, 19, 21, 24, 26
Gemcitabine [50 mg/kg]	d 7, 11, 14, 18, 21, 25

Monitored parameters

- Tumor volume palpated at day 10, 12, 14, 17, 19, 21, 24 after inoculation and at day 26, 28 after harvesting
- Necrospy after harvest at day 26 and 28

Results

No effect of any treatment could be observed by palpation three days after begin of treatment. Strong anti-tumor effect was observed after one week and at day 24 by palpation, with the following ranking in efficacy: Gemzar-50 Taxol < MBT-0206 < MBT-0206 + Gemzar. However, after harvest at day 26, the measured tumor volumes of all groups were clearly lower compared to day 24. This difference in size is most likely due to imprecise palpation before harvest. At day 24 tumor size is reduced to ~ 30% by the mono treatments compared to the control group (n =2). The combination of MBT-0206 + Gemzar resulted in the strongest reduction of the tumor size to 13 %, which was significantly ($p < 0.05$) more effective compared to Taxol, Gemzar and MBT-0206 alone. At day 28 the control group (n =2) is not shown because one of the two tumors was extremely small compared all other tumors (day24, 26, 28) and thus considered as not representative. The tumors after the mono treatments showed a weak increase in tumor size compared to day 26 (Taxol: 536 mm^3; MBT-0206: 392 mm^3; Gemzar: 398 mm^3), whereas the combination therapy led to a slight tumor regression between day 26 and 28 to 88 mm^3.

These data show a strong anti-tumor efficacy of Taxol, Gemzar and MBT-0206 in this model. The anti-tumor action of MBT-0206 is slightly stronger than Taxol but similar to Gemzar. The combination of MBT-0206 and Gemzar shows an impressive anti-tumor efficacy.

9. Anti-tumor efficacy of MBT-0206 in combination with gemcitabine (100 mg/kg) in L3.6pl pancreatic tumors

Treatment

Treatment start: Day 8 after tumor inoculation (01.05.03)

Last treatment: Day 26 after tumor inoculation (19.05.03)

Schedule: MBT-0206, Taxol, trehalose: d 9, 12, 14, 16, 19, 21, 23, 26
Gemcitabine: d 8, 12, 15, 19, 22, 26
Combination: combined mono treatments

Groups (n = 9)	Treatment
Trehalose, Taxol, MBT-0206	d 9, 12, 14, 16, 19, 21, 23, 26
Gemcitabine [100 mg/kg]	d 8, 12, 15, 19, 22, 26
MBT-0206 [5 mg /kg bw] + Gemcitabine [100 mg/kg]	d 9, 12, 14, 16, 19, 21, 23, 26 d 8, 12, 15, 19, 22, 26

Monitored parameters

- Tumor volume palpated at day 23, 26 after inoculation and after harvesting
- Body weight from day 1, 7 ,12, 16, 19, 21, 23, 27
- Necrospy after harvest at day 27

Results (see figures 1 and 2)

Clear anti-tumor effect of all therapeutic treatments was observed at day 23 after tumor inoculation, with an prominent efficacy of the combination therapy (fig. 1). Ranking of tumor inhibition: Taxol < MBT-0206 = Gemzar-50 < MBT-0206 + Gemzar. Compared to the control group, the final tumor volumes were significantly reduced by MBT-0206 to 46% ($p<0.05$), by Gemzar to 47% ($p<0.01$) and by the combination therapy to 22% ($p<0.01$) at day 27. Taxol treatment reduced the final tumor volume to 68%, which was not significant. Interestingly, the efficacy of MBT-0206 and the combination therapy were more pronounced at day 23. Responsible for that might be the extended therapeutic interval during the weekend between day 23 and 27. These data

reveal a clear anti-tumor efficacy of Taxol, Gemzar and MBT-0206 in this model. The anti-tumor action of MBT-0206 is slightly stronger than Taxol but similar to Gemzar. Both MBT-0206 and the combination with Gemzar inhibited the formation of metastases (fig. 2). Liver metastases were absent only in the these two groups. Additionally, only in the combination group the lymph node metastases were rare. The data revealed that the combination of MBT-0206 and Gemzar enhances the anti-tumor efficacy of either mono-therapy.

10. Killing of paclitaxel resistant cells (e.g. tumor cell lines)

To demonstrate the potential of MBT-0206 to directly kill tumors expressing (multi) drug resistance, two highly paclitaxel resistant mammalian tumor cell lines were investigated in vitro. These cell lines were selected by stepwise increasing the concentration of Taxol® in the culture medium. Both cell lines have developed a high resistance level which is reflected by concentrations for 50 % growth inhibition (IC50 value) for Taxol® around 1 or 5 µM (867 or 5000 ng/ml). In both instances, MBT-0206 is clearly superior to Taxol® in killing drug resistant tumor cells. In contrast, in drug-sensitive or low-resistant cell lines, MBT-0206 has a more or less identical killing potential to Taxol®.

MBT-0206 and the human uterus sarcoma derived cell line Mes-SA and its derivative lines

The highly paclitaxel resistant derivative cell line Mes-SA/Dx-5_{MBT} was selected with increasing Taxol® concentrations from the commercially available line Mes-SA/Dx-5 (ATCC, [Harker, 1986 #29]). As shown in Fig. 3, [illegible] resistance to paclitaxel, indicated by the IC50 value of 867 ng/ml.

level of cross resistance for paclitaxel (compare Figs. 3 - 5). The IC50 value for Taxol® is approximately 7-fold lower than in Mes-SA/Dx-5_{MBT}. Concomitantly, there is only a slight tendency of higher killing potential of MBT-0206 compared to Taxol® in this cell line (Fig. 4). The parental line Mes-SA is highly sensitive for Taxol® indicated by the low IC50 value of 5.5 ng/ml (Fig. 5). Against this drug-sensitive line, MBT-0206 has the same killing potential as Taxol®. This is also true for all other paclitaxel-sensitive lines investigated so far. As example for this notion the results of treatments with MBT-0206 and Taxol® of the immortalised endothelial line EA.hy926 are shown in Fig. 8.

MBT-0206 and the murine colon carcinoma derived cell line Colon-26

In a similar way to Mes-SA/Dx-5_{MBT}, a highly paclitaxel resistant derivative line of the murine colon carcinoma line Colon-26 (Cell lines Service, Heidelberg) was established and called Colon-26_{MBT}. The IC50 value for Taxol® is approximately 5 µg/ml (Fig. 6). Again as in Mes-SA/Dx-5_{MBT}, MBT-0206 had a clearly higher potential to inhibit the growth of this cell line. In this cell line, the IC50 values differ by a factor of 3. In line with the result shown for Mes-SA and EA.hy926 cells, the parental drug-sensitive line Colon-26 is equally sensitive for MBT-0206 and Taxol® (Fig. 7).

Conclusion

In highly paclitaxel-resistant cell lines, MBT-0206 has a significantly higher killing potential as Taxol®. In paclitaxel-sensitive lines, both paclitaxel formulations have a comparable efficacy. MBT-0206 may therefore be able to kill also (multi) drug resistant tumors directly in vitro and in vivo. It may, therefore, be a new approach to treat human tumors (or other diseases) which become unresponsive for paclitaxel.

Reference List

1. Rowinsky, E.K., and R.C. Donehower. 1995. Paclitaxel (taxol). *N Engl J Med* 332:1004-1014.
2. Awada, A. 2002. New cytotoxic agents and molecular-targeted therapies in the treatment of metastatic breast cancer. *Forum (Genova)* 12:4-15.
3. Seidman, A.D. 2003. Monotherapy options in the management of metastatic breast cancer. *Semin Oncol* 30:6-10.
4. Romanini, A., L. Tanganelli, F. Carnino, A. Fanucchi, R. Lionetto, S. Pastorino, S. Cosio, A. Gadducci, and P.F. Conte. 2003. First-line chemotherapy with epidoxorubicin, paclitaxel, and carboplatin for the treatment of advanced epithelial ovarian cancer patients. *Gynecol Oncol* 89:354-359.
5. Blom, R., N. Palm, and E. Simonsen. 1996. Paclitaxel (Taxol) monotherapy in the treatment of progressive and recurrent ovarian carcinoma after platinum-based chemotherapy. *Acta Oncol* 35:733-736.
6. Modi, S., K.S. Panageas, E.T. Duck, A. Bach, N. Weinstock, J. Dougherty, L. Cramer, C. Hudis, L. Norton, and A. Seidman. 2002. Prospective exploratory analysis of the association between tumor response, quality of life, and expenditures among patients receiving paclitaxel monotherapy for refractory metastatic breast cancer. *J Clin Oncol* 20:3665-3673.
7. Ozols, R.F., B.N. Bundy, B.E. Greer, J.M. Fowler, D. Clarke-Pearson, R.A. Burger, R.S. Mannel, K. DeGeest, E.M. Hartenbach, and R. Baergen. 2003. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III

8. Vogelstein, B., E.R. Fearon, S.R. Hamilton, S.E. Kern, A.C. Preisinger, M. Leppert, Y. Nakamura, R. White, A.M. Smits, and J.L. Bos. 1988. Genetic alterations during colorectal-tumor development. *N Engl J Med* 319:525-532.

9. Kerbel, R.S. 1991. Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. *Bioessays* 13:31-36.

10. Schünemann, Possinger, Scheidel, and Willich. 1999. Gynäkologische Malignome. Zuckschwerdt GmbH, Germering/München.

11. Heidemann, E., B. Steinke, and H.D. Waller. 1997. Therapieschemata Onkologie und Hämatologie. Urban & Schwarzenberg, München.

12. Heinemann, V. 2003. Role of gemcitabine in the treatment of advanced and metastatic breast cancer. *Oncology* 64:191-206.

13. Thigpen, J.T., J.A. Blessing, G. Olt, S.S. Lentz, and J. Bell. 2003. Cisplatin as second-line therapy in ovarian carcinoma treated initially with single-agent paclitaxel: a Gynecologic Oncology Group study. *Gynecol Oncol* 90:581-586.

14. Kuenen, B.C., L. Rosen, E.F. Smit, M.R. Parson, M. Levi, R. Ruijter, H. Huisman, M.A. Kedde, P. Noordhuis, W.J. van der Vijgh, G.J. Peters, G.F. Cropp, P. Scigalla, K. Hoekman, H.M. Pinedo, and G. Giaccone. 2002. Dose-finding and pharmacokinetic study of cisplatin, gemcitabine, and SU5416 in patients with solid tumors. *J Clin Oncol* 20:1657-1667.

15. Sledge, G.W., Jr. 2003. Gemcitabine combined with paclitaxel or paclitaxel/trastuzumab in metastatic breast cancer. *Semin Oncol* 30:19-21.

16. Reck, M., J. von Pawel, H.N. Macha, E. Kaukel, K.M. Deppermann, R. Bonnet, K. Ulm, S. Hessler, and U. Gatzemeier. 2003. Randomized phase III trial of paclitaxel, etoposide, and carboplatin versus carboplatin, etoposide, and vincristine in patients with small-cell lung cancer. *J Natl Cancer Inst* 95:1118-1127.

17. Zimpfer-Rechner, C., U. Hofmann, R. Figl, J.C. Becker, U. Trefzer, I. Keller, A. Hauschild, and D. Schadendorf. 2003. Randomized phase II study of weekly paclitaxel versus paclitaxel and carboplatin as second-line therapy in disseminated melanoma: a multicentre trial of the Dermatologic Co-operative Oncology Group (DeCOG). *Melanoma Res* 13:531-536.

18. Sledge, G.W., D. Neuberg, P. Bernardo, J.N. Ingle, S. Martino, E.K. Rowinsky, and W.C. Wood. 2003. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). *J Clin Oncol* 21:588-592.

19. Nobmann, S., B. Bauer, and G. Fricker. 2001. Ivermectin excretion by isolated functionally intact brain endothelial capillaries. *Br J Pharmacol* 132:722-728.

20. Thomas, H., and H.M. Coley. 2003. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control* 10:159-165.

21. Harker, W.G., and B.I. Sikic. 1985. Multidrug (pleiotropic) resistance in doxorubicin-selected variants of the human sarcoma cell line MES-SA. *Cancer Res* 45:4091-4096.

22. Fellner, S., B. Bauer, D.S. Miller, M. Schaffrik, M. Fankhanel, T. Spruss, G. Bernhardt, C. Graeff, L. Farber, H. Gschaidmeier, A. Buschauer, and G. Fricker. 2002. Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. *J Clin Invest* 110:1309-1318.

23. Kiesewetter, D.O., E.M. Jagoda, C.H. Kao, Y. Ma, L. Ravasi, K. Shimoji, L.P. Szajek, and W.C. Eckelman. 2003. Fluoro-, bromo-, and [illegible] synthesis and biological evaluation. *Nucl*

25. Leonard, G.D., O. Polgar, and S.E. Bates. 2002. ABC transporters and inhibitors: new targets, new agents. *Curr Opin Investig Drugs* 3:1652-1659.

26. Agrawal, M., J. Abraham, F.M. Balis, M. Edgerly, W.D. Stein, S. Bates, T. Fojo, and C.C. Chen. 2003. Increased 99mTc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin Cancer Res* 9:650-656.

27. Callies, S., D.P. de Alwis, A. Harris, P. Vasey, J.H. Beijnen, J.H. Schellens, M. Burgess, and L. Aarons. 2003. A population pharmacokinetic model for paclitaxel in the presence of a novel P-gp modulator, Zosuquidar Trihydrochloride (LY335979). *Br J Clin Pharmacol* 56:46-56.

28. Lindl, T., and J. Bauer. 1994. Zell- und Gewebekultur. Gustav Fischer Verlag, Stuttgart.

29. Harker, W.G., D. Bauer, B.B. Etiz, R.A. Newman, and B.I. Sikic. 1986. Verapamil-mediated sensitization of doxorubicin-selected pleiotropic resistance in human sarcoma cells: selectivity for drugs which produce DNA scission. *Cancer Res* 46:2369-2373.

Claims

1. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for administering to a human patient in need thereof at a monthly dose of about 0.25 mg up to about 60 mg of paclitaxel / kg body weight of said patient.

2. The use of claim 1, wherein said monthly dose is about 0.5 mg up to about 30 mg paclitaxel / kg body weight.

3. The use of claim 1 or 2, wherein said monthly dose is about 1.0 mg up to about 15 mg paclitaxel / kg body weight.

4. The use of any one of the claims 1 to 3, wherein administering said cationic liposomal preparation is at least once a time daily.

5. The use of any one of the claims 1 to 4, wherein administering said cationic liposomal preparation is a plurality of times during a month period, each of said times being separated by an interval of between one day and 3 weeks.

6. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryotherapy.

7. The use of any one of the claims 1 to 6, wherein said cationic liposomal preparation comprises paclitaxel in an amount of at least about 2 mole% to about 8 mole%.

8. The use of any one of the claims 1 to 7, wherein said cationic liposomal preparation comprises paclitaxel in an amount of about 2.5 mole% to about 3.5 mole%.

9. The use of any one of the claims 1 to 8, wherein said cationic liposomal preparation comprises 50:47:3 mole% of DOTAP, DOPC and paclitaxel.

10. The use of any one of the claims 1 to 9, wherein said cationic liposomal preparation comprises substantially no paclitaxel crystals.

11. The use of any one of the claims 1 to 10 for treating an angiogenesis-associated condition.

12. The use of claim 11 for treating wound healing, cancer, an inflammatory disease or a chronic inflammatory disease such as rheumatoid arthritis, dermatitis, psoriasis or endometriosis.

13. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for the prevention or treatment of disorders associated with and/or accompanied by the occurrence of drug resistant cells, e.g. for the prevention or treatment of drug-resistant tumors.

14. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for the prevention or treatment of metastasis formation, e.g. onset and/or progression, particularly associated with and/or accompanied by a tumor disorder.

15. The use of claim 14 for manufacturing a pharmaceutical composition for the prevention or treatment of liver metastasis formation.

16. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryotherapy against metastasis onset and/or progression, e.g. associated with and/or accompanied by the tumors.

17. The use of any one of the claims 13 to 16, wherein said active agent is selected from a cytotoxic or cytostatic substance such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent or an immunological active substance.

18. The use of claim 17, wherein said first active agent is selected from a taxane, a camptothecin, a statin, a depsipeptide, thalidomide, other agents interacting with microtubuli such as discodermolide, laulimalide, isolaulimalide, eleutherobin, Sarcodictyin A and B, and in a most preferred embodiment it is selected from paclitaxel, docetaxel, camptothecin or any derivative thereof.

19. The use of claim 6 or 16, wherein said second active agent is an anti-endothelial cell active substance, an anti-tumor active substance, a chemotherapeutic agent, an immunological active substance, a compound that reduces or eliminates hypersensitivity reactions or a chemosensitizer.

20. The use of claims 17, 18 or 19, wherein said active agent is selected from antineoplastic agents such as: antimitotic agents like paclitaxel (Taxol), alkylating agents such as platinum containing compounds like cisplatin, carboplatin, DNA topoisomerase inhibiting agents like camptothecin or doxorubicin, RNA / DNA antimetabolites such as 5-fluorouracil or gemcitabine and other compounds having antitumor activity.

21. The use of claim 19, wherein said compound that reduces or eliminates hypersensitivity reactions is selected from the group comprising steroids, antihistamines, H2 receptor antagonists, and combinations thereof in a sufficient amount to prevent fatal anaphylactic reactions.

22. The use of claim 21, wherein said compound is selected from the group comprising Ranitidine, Dexamethasone, Diphenhydramine, Famotidine, Hydrocortisone, Clemastine, Cimetidine, Prednisolone, Chlorpheniramine, Chlorphenamine, Dimethindene maleate, and Promethazine.

23. The use of claim 19, wherein said chemosensitizer is selected from the

24. The use of any one of the claims 1 to 23, wherein said cationic liposomal preparation comprises liposomes having an average particle diameter from about 50 nm to about 500 nm, preferably about 100 nm to about 300 nm

25. The use of any one of the claims 1 to 24, wherein said cationic liposomal preparation is administered systemically, preferably intravenously.

Abstract

The present invention relates to the use of pharmaceutical preparations comprising paclitaxel for administration to a human patient in need thereof.

kt/ANM/30852PEP-15.10.2003

Figure 1

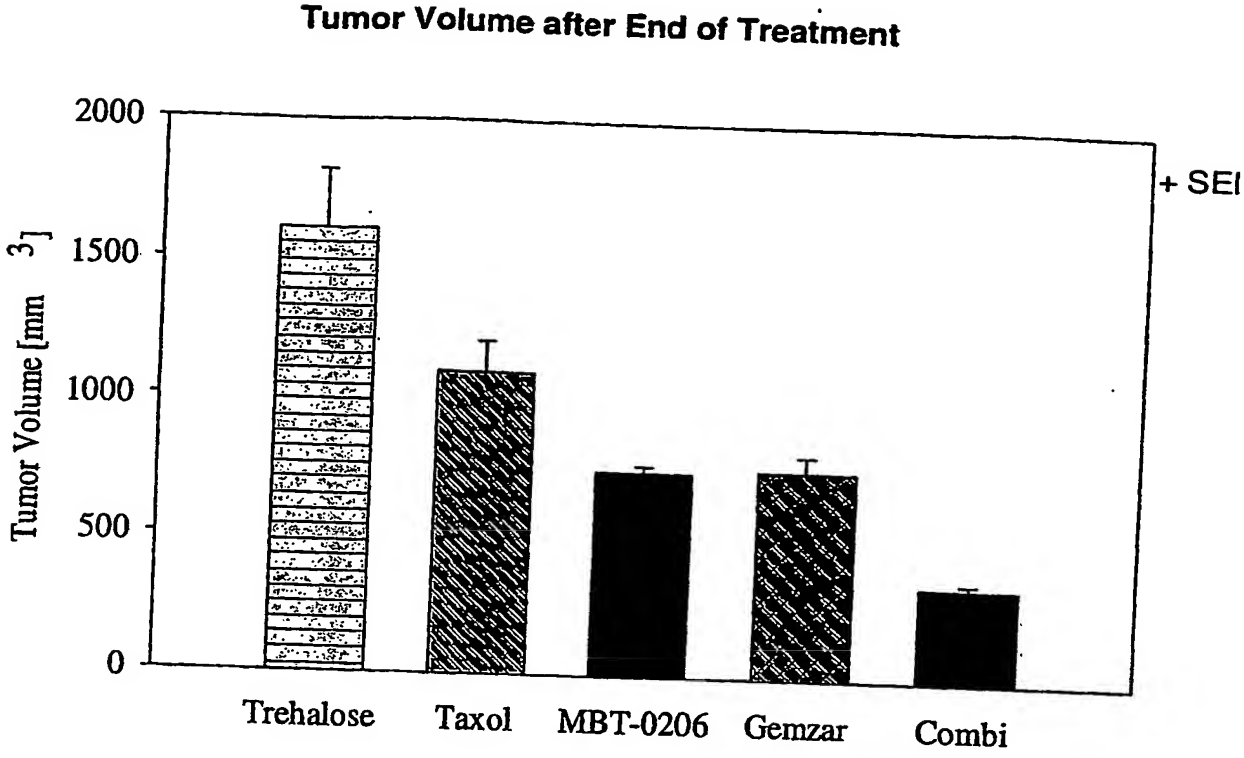

Figure 2

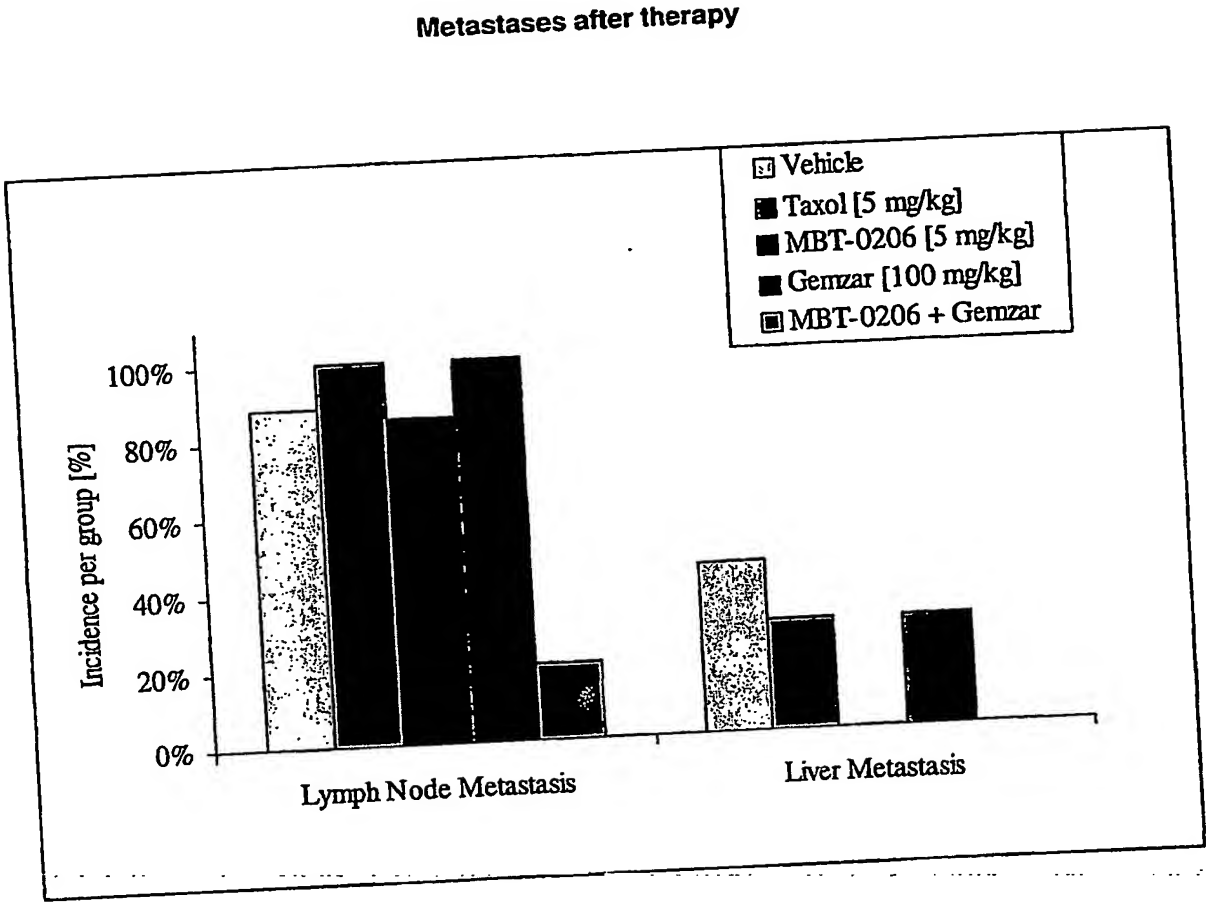
Metastases after therapy
Vehicle
Taxol [5 mg/kg]
MBT-0206 [5 mg/kg]
Gemzar [100 mg/kg]
MBT-0206 + Gemzar
Incidence per group [%]
100%
80%
60%
40%
20%
0%
Lymph Node Metastasis
Liver Metastasis

Figure 3

Inhibitory Potential of MBT-0206 and Taxol® against the highly drug-resistant uterus sarcoma line Mes-SA/Dx-5$_{MBT}$

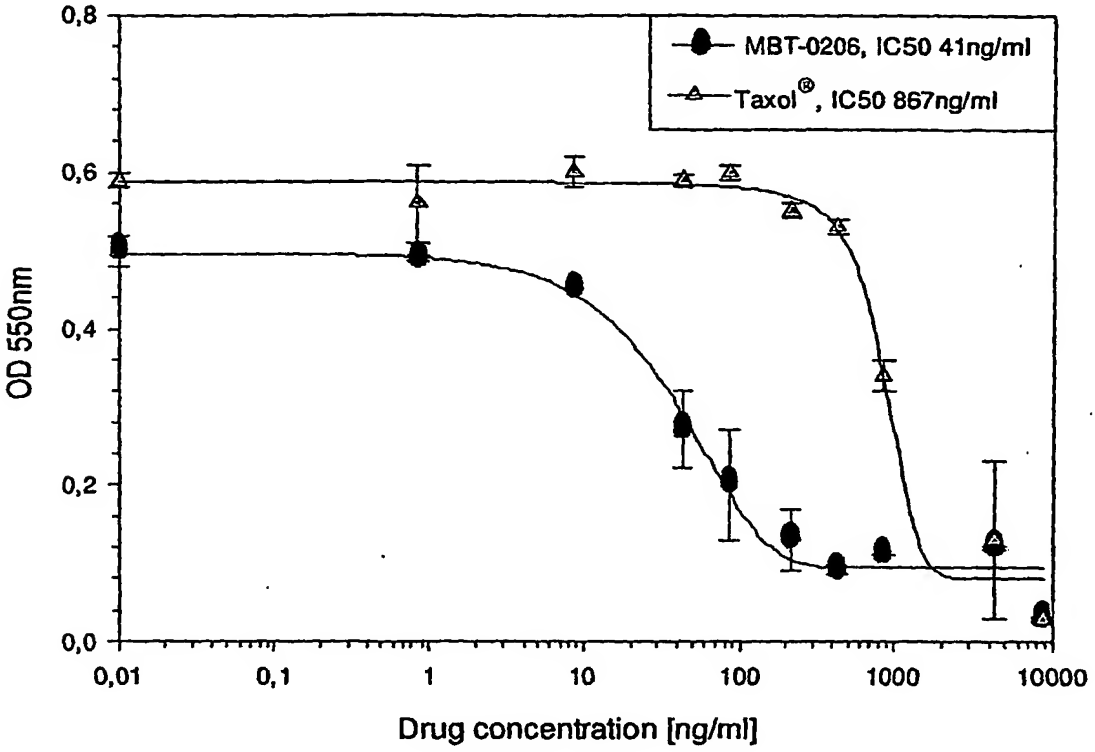

Figure 4

Inhibitory Potential of MBT-0206 and Taxol® against the moderatly drug-resistant uterus sarcoma line Mes-SA/Dx-5

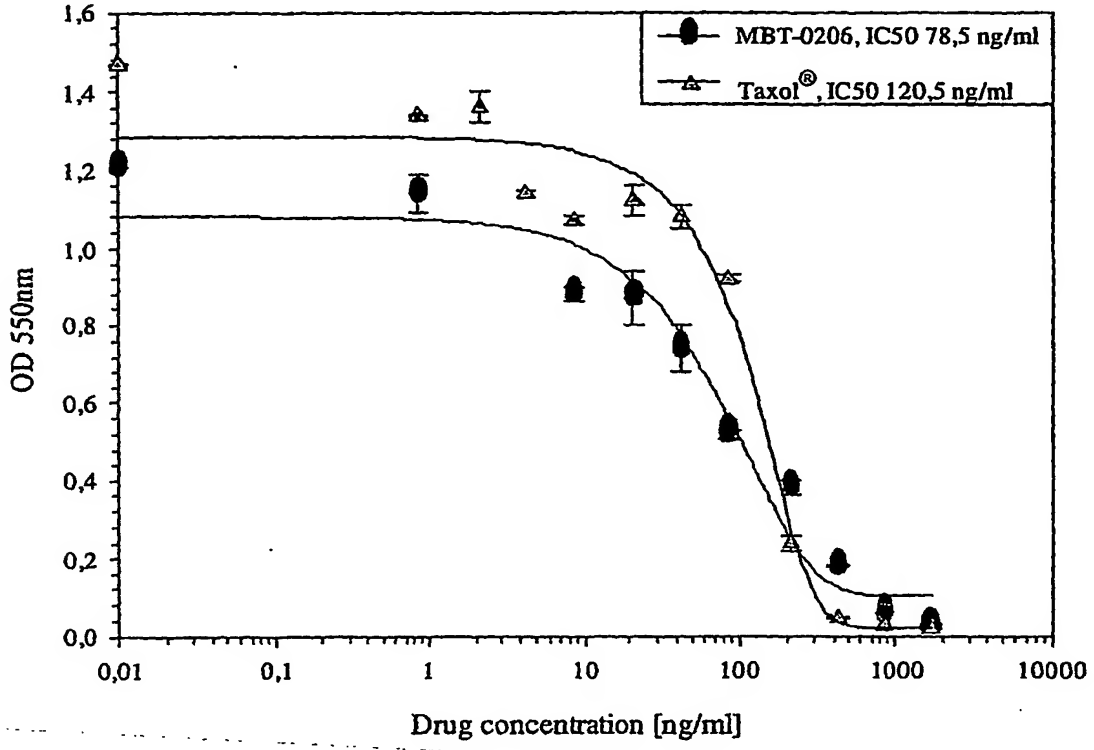

Figure 5

Inhibitory Potential of MBT-0206 and Taxol® against the drug-sensitive human uterus sarcoma line Mes-SA

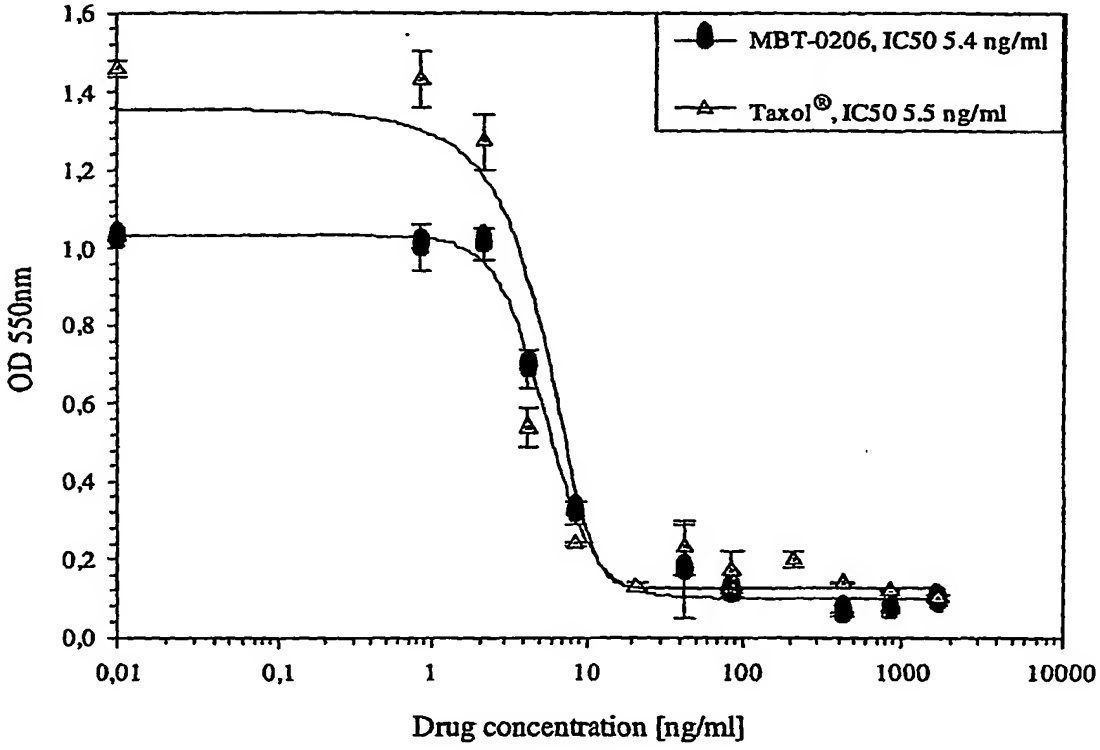

Figure 6

Inhibitory Potential of MBT-0206 and Taxol® against the highly drug-resistant murine colon carcinoma line Colon-26$_{MBT}$

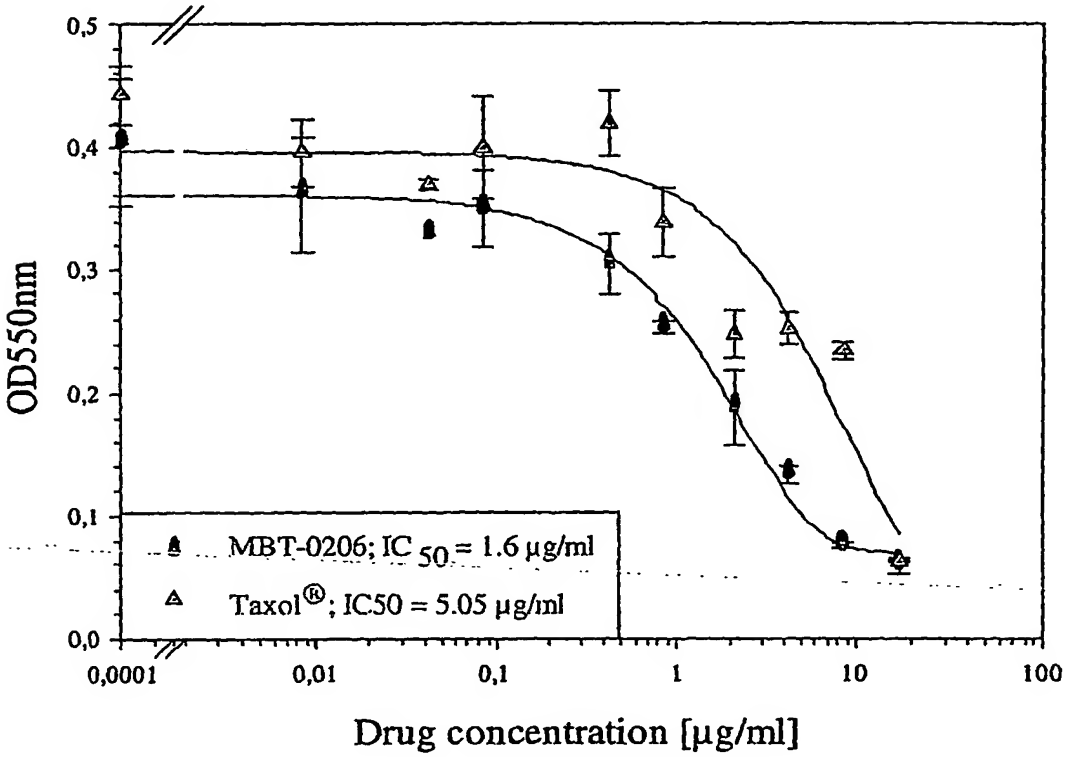

Figure 7

Inhibitory Potential of MBT-0206 and Taxol® against the parental drug-sensitive murine colon carcinoma line Colon-26

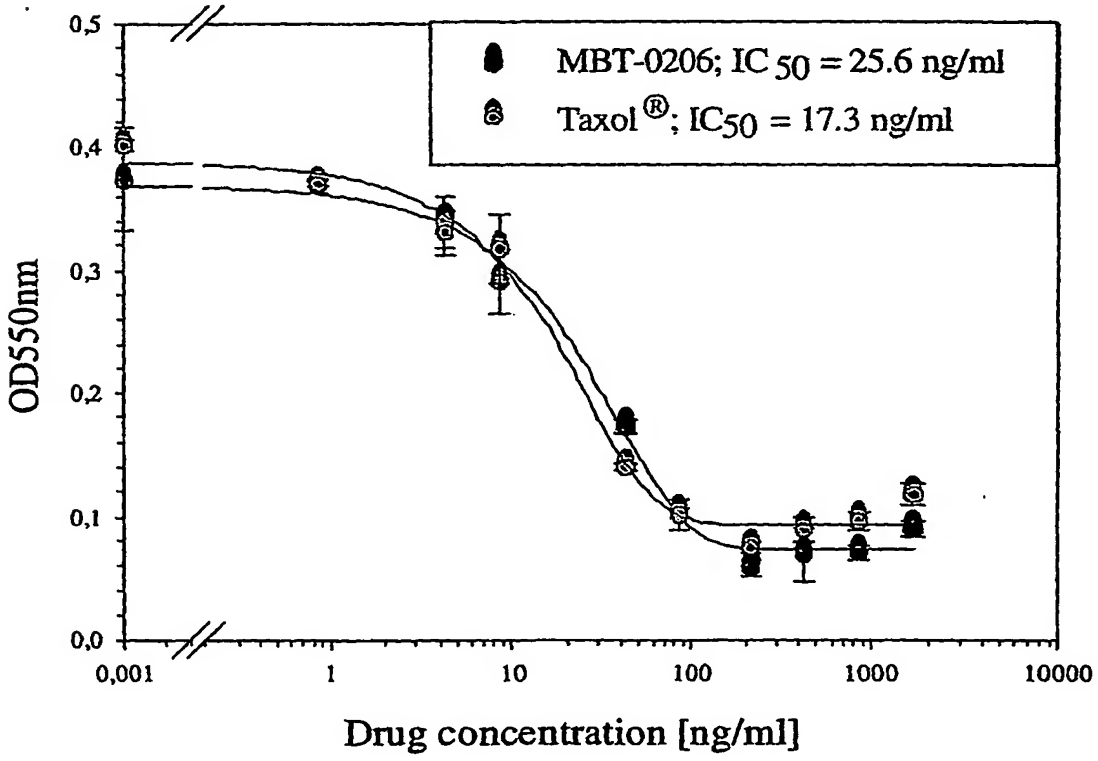

Figure 8

Inhibitory Potential of MBT-0206 and Taxol® against the drug-sensitive human endothelial line EA.hy926